CHAPTER 9

FLUOROQUANT TEST METHOD

9.1 GENERAL INFORMATION

The Romer Fluoroquant aflatoxin test method uses fluorescence technology to quantitatively measure total aflatoxins (B1, B2, G1, and G2) in parts per billion (ppb).

9.2 PREPARATION OF SOLUTIONS

a. Developer Solution.

Prepare the working developer reagent by adding 50 ml of deionized or distilled water to the bottle supplied with the repipettor and then adding the contents of one ampule of developer concentrate.

To insure complete and accurate transfer, rinse the ampule three times with the working developer solution, each time returning the rinse to the bottle. Swirl to mix contents.

There is room in the repipettor bottle to make a larger amount of developer by adding the contents of 2 ampules to 100 ml of water.

Initially prime the repipettor in a waste tube to remove any air bubbles. When using the repipettor, pull up the plunger all the way to the stop, then push all the way back down to insure accurate delivery volume. Before each use, prime the repipettor slightly to remove any air that may form at the tip. Place the cap on the end of the repipettor tip after each use.

NOTE: The developer working reagent must be made fresh every 8 hours.

b. 80 Percent Methanol Solution **(for corn, corn meal, rice, popcorn, sorghum, and wheat)**.

(1) Using a graduated cylinder, measure 800 ml of methanol (HPLC grade) and place it into a clean carboy with spigot.

(2) Add 200 ml deionized or distilled water to the methanol and shake vigorously until it is completely mixed.

(3) Label the container stating the mixture (80 percent methanol and 20 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 8 parts methanol to 2 parts of deionized or distilled water.

c. 90 Percent Acetonitrile Solution **(for soybeans and corn/soy blend)**.

(1) Using a graduated cylinder, measure 900 ml of acetonitrile and place it into a clean carboy with spigot.

(2) Add 100 ml deionized or distilled water.

(3) Label the container stating the mixture (90 percent acetonitrile and 10 percent water), date of preparation, and initials of technician who prepared the solution.

(4) Store this solution at room temperature in a tightly closed container until needed.

NOTE: To prepare smaller or larger amounts of solution use the ratio of 9 parts acetonitrile to 1 part of deionized or distilled water.

9.3 FLUOROMETER CALIBRATION

a. Turn the power on and respond as indicated (no warm-up period is required).

b. When first turned on, the fluorometer will go through a series of self-tests. After the self-tests are completed, the screen will allow you to change the date and time, or continue. You will then come to the "METHOD" screen. This screen allows you to choose a particular method, print a list of methods, or set up a new method. If the fluorometer has not yet been programmed with the appropriate method, use the following directions.

(1) Press the "Set Up" key.

(2) Enter the method number according to the sample matrix being tested and press "Enter".

(3) Enter the delay time as 40 seconds.

(4) Select the measurement as "ppb".

(5) Select the results as "decimals".

(6) Enter the **High Calibrator** value according to the calibration card provided.

(7) Enter the **Low Calibrator** value as stated and continue with the procedure.

c. Press the "Select" key.

d. Enter the method number according to the sample matrix being tested and press "Enter".

e. After choosing the method desired, place the high and low calibrator ampules in the fluorometer when specified.

f. For the first sample, insert the control into the fluorometer and read the result. The control range will read as stated on the control ampule bottle. If the reading is not in this range, try recalibrating the machine. If the control value is still out of range, contact the Technical Services Department at Romer Labs Inc. at 1-800-769-1380.

9.4 EXTRACTION PROCEDURES

a. Transfer 50 grams of ground sample into an extraction mixing jar.

b. Add 100 ml of the extraction solvent.
(methanol/ water for corn, corn meal, rice, popcorn, sorghum, and wheat)
(acetonitrile/water for soybeans and corn/soy blend)

c. Cover the extraction jar and blend on high speed for 1 minute.

d. Remove the cover and funnel the extract through a Whatman No.1 filter or a coffee filter into a sample jar labeled with the sample identification.

e. After collecting the filtrate, remove the funnel, filter, and ground material and place over an empty collection container (e.g., disposable plastic beaker).

9.5 TEST PROCEDURES

a. Purification of Corn, Corn Meal, Rice, Popcorn, Sorghum, and Wheat.

(1) Make sure the clear plastic tip is pushed firmly onto the bottom of the UniSep 2001 column. This prevents any solution from passing through the column prematurely.

(2) Place 1 ml of the extract in the top of the column and discard the pipette tip.

(3) Add 1 ml of the diluent and discard the pipette tip.

(4) Place the blue cap on top of the column and mix thoroughly by hand, shaking vigorously for 5 seconds.

(5) Uncap the top and bottom of the column and place the column in a 12 x 75 mm cuvette. Insert the syringe barrel and stopper into the top of the column.

(6) Slowly **(30-40 seconds)** push the extract through the column until air comes out of the bottom.

NOTE: It is critical to push the solution completely through the column in at least a 30-40 second time-frame indicated to insure a complete extraction solution purification.

(7) Transfer 0.5 ml of each purified sample extract to a clean 12 x 75 mm cuvette and cap.

b. Purification of Soybeans and Corn/Soy Blend.

(1) Make sure the clear plastic tip is pushed firmly onto the bottom of the UniSep 2001 column. This prevents any solution from passing through the column prematurely.

(2) Place 2 ml of the extract in the top of the column and discard the pipette tip.

(3) Place the blue cap on top of the column and mix thoroughly by hand, shaking vigorously for 5 seconds.

(4) Uncap the top and bottom of the column and place the column in a 12 x 75 mm cuvette. Insert the syringe barrel and stopper into the top of the column.

(5) Slowly **(30-40 seconds)** push the extract through the column until air comes out of the bottom.

NOTE: It is critical to push the solution completely through the column in at least a 30-40 second time-frame indicated to insure a complete extraction solution purification.

(6) Transfer 250 µl (0.25 ml) of each purified sample extract to a clean 12 x 75 mm cuvette.

(7) Add 250 µl of methanol to the cuvette and cap.

c. Derivatization and Fluorometric Reading.

(1) Immediately add 1 ml of the developer working reagent to each purified sample.

(2) Recap the tube and vortex for 5 seconds.

(3) Wipe the cuvette with lint-free paper and place in the fluorometer for a reading.

(4) After a 40-second delay, the result will appear on the fluorometer screen and a record will be printed out.

NOTE: Once the developer reagent is added, the sample must be mixed, and the sample cuvette must be placed in the fluorometer quickly. Samples must be derivatized one sample at a time and then read before proceeding to the next sample.

9.6 REPORTING AND CERTIFYING TEST RESULTS

a. Record the digital readout as ppb total aflatoxins in the sample.

b. Report all results on the pan ticket and the inspection log to the nearest whole ppb.

c. Sample results over 300 ppb are reported as >300 ppb unless a supplemental analysis is performed.

d. Refer to the Certification section of the handbook for more detailed certification procedures.

9.7 SYSTEM CHECK

a. Positive Control Option.

The test kit contains a positive control standard that may be used as a check on method technique and overall system performance.

b. System Check Procedures.

(1) Make sure the clear plastic tip is pushed firmly onto the bottom of the UniSep2001 column.

(2) Place 1 ml of **80/20 methanol/water solution** in the top of the column and discard the pipette tip.

Note: Do not use the 90/10 acetonitrile/water solution in this procedure.

(3) Add 1 ml of Positive Control Standard and discard the pipette tip.

(4) Place the blue cap on top of the column and mix thoroughly by hand shaking vigorously for 5 seconds.

(5) Uncap the top and bottom of the column and place the column in a 12 x 75 mm cuvette. Insert the syringe barrel and stopper into the top of the column.

(6) Slowly **(30-40 seconds)** push the extract through the column until air comes out the bottom.

(7) Transfer 0.5 ml of the purified positive control solution to a clean 12 x 75 mm cuvette and cap.

(8) Immediately add 1 ml of developer and working reagent to the positive control standard.

(9) Recap the tube and vortex for 5 seconds.

(10) Wipe the cuvette with lint-free paper and place in the fluorometer for a reading.

(11) After a 40-second delay, the result will appear on the fluorometer screen and a record will be printed out. The value received should fall within the range listed on the label of the bottle.

NOTE: Be sure to use the corn calibration factors to test the control standard.

9.8 SUPPLEMENTAL ANALYSIS

a. Diluting the Sample Extract.

If quantitative results are above the test method's conformance limit, test results are reported as exceeding the limit. To determine and report an aflatoxin level higher than 300 ppb, the sample extract must be diluted so that a value between 5 and 300 ppb is obtained. The final aflatoxin concentration is calculated by multiplying the results with the diluted extract by the dilution factor.

b. Example.

If the original analysis reported the aflatoxin value at 700 ppb, the sample extract would be diluted using the following procedures in order to obtain a true value.

(1) Dilute 5 ml of the original extract with 10 ml of the extraction solvent mixture **(methanol/ water for corn, corn meal, rice, popcorn, sorghum, and wheat**, **acetonitrile/water for soybeans and corn/soy blend).** The total volume is 15 ml. This is a 1 to 3 dilution (compares volume in the beginning with the total volume in the end).

(2) Multiply the analytical results obtained by 3 to obtain the actual aflatoxin concentration. For example, if 240 ppb was the original value obtained with the diluted extract, the actual concentration in the original sample was 720 ppb.

$$\text{True Aflatoxin Value} = \frac{\text{Total Volume}}{\text{Initial Extract Volume}} \times \text{Aflatoxin Result}$$

True Aflatoxin Value = (15) 5) x 240 ppb
= 3 x 240 ppb = 720 ppb

9.9 CLEANING LABWARE

a. Negative Tests (# 20 ppb).

(1) Labware.

Prepare a solution consisting of dishwashing liquid and water. Completely submerge the used glassware, funnels, beakers, etc., wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Place materials in a garbage bag for routine trash disposal.

b. Positive Tests (> 20 ppb).

(1) Labware.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water (e.g., 100 ml bleach to 1,000 ml water). Completely submerge the used glassware, funnels, beakers, etc., and soak for at least 5 minutes. Remove items from the bleach/water solution, submerge in a dishwashing liquid/water solution, wash thoroughly, then rinse with clean water before reusing.

(2) Disposable Materials.

Prepare a bleach solution consisting of 1 part bleach to 10 parts water in a plastic pail labeled "bleach solution". Soak disposable materials, such as used columns, cuvettes, vials, test kit components, etc., for at least 5 minutes. Pour off the liquid down the drain and place the materials in a garbage bag and discard.

9.10 WASTE DISPOSAL

a. Negative Results (# 20 ppb).

If the test result is negative (equal to or less than 20 ppb), discard the filter paper and its contents (ground material) into a plastic garbage bag for disposal. Dispose of any remaining liquid filtrate in the chemical waste container.

b. Positive Results (> 20 ppb).

If the result is positive (more than 20 ppb), the ground portion remaining in the filter paper must be decontaminated prior to disposal. After disposing of the remaining filtered extract in the chemical waste container, filter approximately 50 ml of bleach through the filter containing the ground portion and allow to drain. Discard the filter paper and its contents (ground portion) into a plastic garbage bag for disposal. The bleach rinse filtrate collected may be treated as a non-hazardous solution and disposed of by pouring down the drain.

9.11 EQUIPMENT AND SUPPLIES

a. Blender with ½ pint jars.

b. Syringe with rubber stopper.

c. Cuvette rack.

e. Pipettor and tips - 200 to 1000 µl (.20 to 1 ml) adjustable.

f. Vortex Mixer.

g. Fluorometer (Vicam Series III or IV, or Romer RL100) and printer.

h. 100 ml graduated cylinder.

i. Funnel.

j. Timer.

k. Whatman No.1 Filter Paper or Coffee Filters.

l. Glass cuvettes (12 x 75 mm).

m. Empty bottles for Developer Working Reagent and Working Diluent.

n. Repipette Dispenser (1ml), Labindustries Model LS830X3 or equivalent.

o. Sample grinder.

p. Balance.

q. HPLC grade Methanol (**for extraction solvent for corn, corn meal, rice, popcorn, sorghum, and wheat).**

r. Acetonitrile **(for extraction solvent for soybeans and corn/soy blend).**

s. Deionized or Distilled Water.

t. UniSep 2001 Fluoroquant "A" columns.

u. Developer Concentrate.

v. Diluent (**for corn, corn meal, rice, popcorn, sorghum, and wheat).**

w. High, Low, and Control calibrator ampules.

x. Positive Control Standard.

9.12 STORAGE CONDITIONS

a. UniSep 2001 columns - Room temperature in a sealed container.

b. Developer Concentrate - shipped in an amber bottle. Store in a tightly closed container in a cool, dry, well ventilated area away from direct sunlight, combustible materials, and incompatible substances.

c. Calibrators - Room temperature.

d. Diluent - In a cool place away from heat source.

e. Positive Control Standard - Refrigerated at 23º to 32º F.